EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) ON BIOLOGY OF GLOMERULAR MESANGIAL AND TUBULAR EPITHELIAL CELLS

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a prototype compound of polyhalogenated aromatic hydrocarbons, is produced during industrial chlorine bleaching of paper pulp, forest fires, waste incineration, automobile exhaust, cigarette smoke, and other combustion process (1). TCDD produces diverse biologic effects including a wasting syndrome, immunotoxicity, hepatotoxicity, and, in case of low-exposure, tumor development and cancer (2). Although fetal hydronephrosis has been observed when pregnant mice were exposed to low doses of TCDD (3,4) and nephrotoxicity of aromatic hydrocarbons such as benzo[*a*]pyrene (BP) is well known (5), little is known about the effects of TCDD on renal function. Thus, the present study examined the effects of TCDD on cell viability, proliferation, and extracellular matrix (ECM) synthesis by glomerular mesangial cells, LLC-PK1 cells representing proximal tubular epithelial cells, and MDCK cells representing distal epithelial cells and compared with the effects of BP. We further examined the role of reactive oxygen species (ROS) on TCDD-induced cellular effects, as previous studies have demonstrated that oxidative stress occurs in various tissues of TCDD-treated animals (6). These include enhanced in vitro and in vivo hepatic and extrahepatic lipid peroxidation, increased hepatic and macrophage DNA damage, increased urinary excretion of malondialdehyde, decreased hepatic membrane fluidity, increased production of superoxide anion by peritoneal macrophage, and deceased glutathione, nonprotein sulfhydryls, and NADPH contents in liver. Oxidative stress plays important role in gene transcription leading to alterations in cell function and ROS are recently viewed as important intracellular signaling molecules (7). Kidney is indeed a susceptible organ to oxidative stress (8) and ROS mediates ECM production of glomerular mesangial cells which may lead to renal injury (9,10).

Methods and Materials

All chemicals and tissue culture plastics were obtained from Sigma Chemical Company (St. Louis, MO, USA) and Nalge Nunc International (Naperville, IL, USA), respectively, unless otherwise stated. Mouse mesangial cells (MMC) were obtained from American Type Cell Collection (ATCC: Rockville, MD, USA) and cultured with DMEM containing 100 U/mL penicillin, 100μ g/mL streptomycin, 26 mM $NaHCO_3$, and 5% fetal bovine serum (FBS). Both LLC-PK1 and MDCK cells were provided by Professor Hitoshi Endo (Department of Pharmacology and Toxicology, Kyorin University School of Medicine, Tokyo, Japan) and cultured with M199 containing 10% FBS and MEM containing 10% FBS, respectively. Quiescent cells were incubated with respective serum free media containing different concentrations of TCDD (1-100 nM), BP (3 and 30μ M), and H_2O_2 (100μ M) for 24-96 hours.

Cell viabiltiy and proliferation were assessed by lactate dehydrogenase (LDH) released from membrane-damaged cells and [^{3}H]-thymidine incorporation, respectively, as previously described (11).

Intracellular ROS production was measured by the method of Bass et al (12). Briefly, cells were washed with Dulbecco's phosphate buffered saline (PBS) and incubated in the dark for 15 min in Krebs-Ringer solution containing 5μ M 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA: Molecular Probes Inc., Eugene, OR). DCFH-DA is a nonpolar compound that readily diffuses into cells, where it is hydrolyzed to the nonfluorescent polar derivative 2',7'-dichlorofluorescin (DCFH) and thereby trapped within the cells (12). In the presence of a proper oxidant, DCFH is oxidized to the highly fluorescent 2',7'-DCF. Cells were detached by use of trypsin and intracellular ROS generation was detected as a result of the oxidation of DCFH (excitation, 488 nm; emission, 515-540 nm) utilizing FACS.

Aliquots of cor ..ioned media were electrophoresed on SDS-PAGE (5% acrylamide), transferred onto a nitrc ellulose membrane, and immunobloted with rabbit anti-human fibronectin antibodies (HRP conjugated, DAKO, Glostrup, Denmark). Positive immunoreactive bands were visualized with ECL detection reagents (Amersham Life Science, Little Chalfont, U.K.) and quantitated densitometrically and compared to controls as previously described (13). Secreted fibronectin protein was normalized by the concentrations of cellular protein measured by the Bradford method (14) using the Bio-Rad assay.

All results are expressed as means±standard error (SE). Analysis of variance with subsequent Fisher's least significant difference method was used to determine significant differences in multiple comparisons. A P value < 0.05 was used as the criterion for a statistically significant difference.

Results and Discussion

LDH release remained unchanged in MMC, LLC-PK1, and MDCK incubated with serum-free media up to 96 hours, suggesting that cell viabilities of all three cell types were not affected under our basal experimental condition. When cells were continuously exposed to TCDD, LDH release significantly increased in MMC, LLC-PK1, and MDCK in a dose- and a time-dependent manner. 100 nM TCDD significantly increased LDH release at 24 hours and 50 nM TCDD at 48 hours. BP up to 30 μ M did not affect LDH release in any cell type, suggesting that TCDD is more cytotoxic than BP.

Effects of TCDD on [^{3}H]-thymidine incorporation were different among MMC, LLC-PK1, and MDCK as summarized in figure 1. [^{3}H]-thymidine incorporation was increased in MMC and LLC-PK1 and decreased in MDCK by TCDD. Reported effects of TCDD on cell proliferation were different among different cells and sometimes contradictory. TCDD induces proliferation in human keratinocytes (15), fetal mouse urinary tract epithelium (16), and rat hepatocytes (17), decreases proliferation in rat hepatocytes (18,19), and abrogates the estrogen-dependent proliferation of human breast cancer cells (20). Since the arylhydrocarbon receptor (AHR), a receptor for TCDD, has been demonstrated to play a major role in regulation of cell cycle control (21), further studies on the expression of AHR in MMC, LLC-PK1, and MDCK may explain the different effects of TCDD on proliferation of three types of cells. Contrary to TCDD, 30μ M BP significantly inhibited [^{3}H]-thymidine incorporation in MMC and MDCK but not in LLC-PK1.

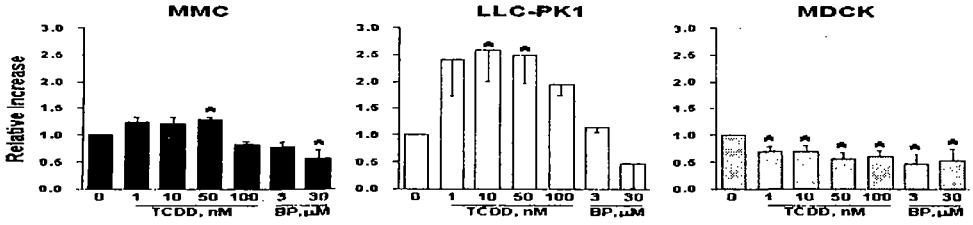

Figure 1. Effects of TCDD and BP on [^{3}H]-thymidine incorporation by MMC, LLC-PK1, and MDCK at 72 hours after stimulation. Quiescent cells were incubated continuously for 72 hours with respective serum-free media containing different concentrations of TCDD or BP. Afterward, thymidine incorporation was measured as described in "Methods". Values are mean±SE of 4-5 experiments. *p<0.05 compared with control.

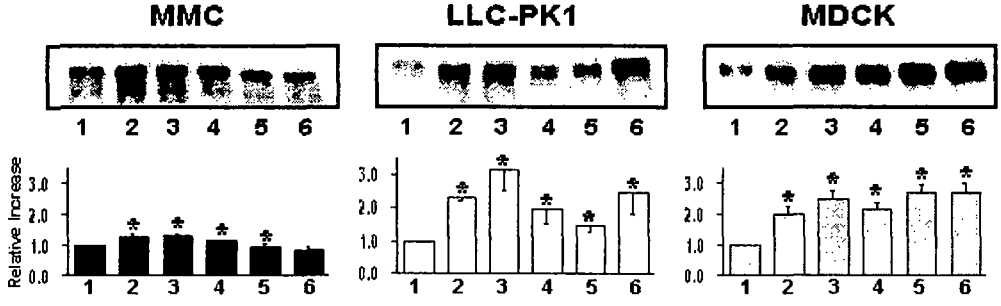

Figure 2. Effects of TCDD and BP on fibronectin secretion by MMC, LLC-PK1, and MDCK at 48 hours after stimulation. Quiescent cells were incubated continuously for 48 hours with respective serum-free media containing 1 and 10 nM TCDD, 3 and 30μ M BP, or 100μ M H_2O_2. Afterward, fibronectin in the media was measured as described in "Methods". Values are mean±SE of 3 experiments. *p<0.05 compared with control. 1:serum free media, 2:1 nM TCDD, 3:10 nM TCDD, 4:3μ M BP, 5:30μ M BP, 6: 100μ M H_2O_2

As summarized in figure 2, both TCDD and BP increased fibronectin secretion by MMC, LLC-PK1, and MDCK cells, suggesting that TCDD and BP may cause renal fibrosis leading to loss of renal function. 1 nM TCDD- and 3μ M BP-induced fibronectin secretion by all three types of cells were effectively inhibited by 500μ M taurine, an antioxidant (data not shown). In addition, H_2O_2 at 100μ M increased fibronectin secretion in MMC, LLC-PK1, and MDCK, and DCF-sensitive intracellular ROS also increased in response to TCDD and BP in all three types of cells (data not shown). These data suggest that both TCDD and BP induce fibronectin synthesis through ROS generation in renal cells.

In conclusion, these data provide experimental evidence that TCDD can alter cell viability and proliferation and increase ECM synthesis by renal cells which may lead to renal injury and that ROS may play an important role in TCDD-induced renal cell activation. The present study also provide a new experimental in vitro model which should prove valuable in evaluating the mechanisms through which TCDD disrupt normal regulation of proliferation and ECM accumulation.

Acknowledgments

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